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PATENT
Attorney Docket No.: A-63487-3/RFT/JJD

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#9B
3etc
5/5/02

In re application of:

SIMMONS et al.

Serial No. 09/829,251

Filed: April 9, 2001

For: METHODS AND COMPOSITIONS FOR SECRETION OF HETEROLOGOUS POLYPEPTIDES

Examiner: UNKNOWN

Group Art Unit: 1636

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on:

Dated: November 30, 2001

Signed: Monica Carlos
Monica E. Carlos

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

This amendment accompanies the response to the Notice to Comply With Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures mailed May 30, 2001, a copy of which is enclosed herewith. The Commissioner is authorized to charge any, including any extension of time fees, or other relief, as may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-63487-3/RFT/JJD).

Please enter the following amendments, as indicated below.

Please cancel the text appearing from page 4, line 33 to page 5, line 22 (paragraphs beginning at page 4, line 33, page 5, line 3 and page 5, line 11) of the specification as originally filed.

Please replace the paragraph beginning at page 19, line 33, with the following amended paragraph:

-- Each 0.5 OD_{600} pellet was then prepared for gel analysis as follows. Each pellet was resuspended in 50 µl TE (10mM Tris pH7.6, 1mM EDTA). After the addition of 10 µl 10% SDS, 5 µl reducing agent (1M dithiothreitol or 1M β-mercaptoethanol), the samples were heated at about 90°C for 2 minutes and then vortexed. Samples were allowed to cool to room temperature, after which 500 µl acetone was added. The samples were vortexed and then left at room temperature for about 15 minutes. Samples were centrifuged for 5 minutes. The supernatants were discarded, and the pellets resuspended in 20 µl water, 5 µl reducing agent, 25 µl NOVEX 2X sample buffer. Samples were heated at about 90°C for 3-5 minutes, then vortexed. After centrifugation for 5 minutes, supernatants were transferred to clean tubes and the pellets discarded. 5-10 µl of each sample was loaded onto 10 well, 1.0 mm NOVEX manufactured gel (San Diego, CA.) and electorphoresed for 1.5-2 hr at 120 volts. Gels were stained with Coomassie blue to visualize polypeptide.--

Please insert the following three paragraphs, preceding the paragraph beginning at page 20, line 11 of the specification as originally filed:

-- In one case, a polypeptide gel was run and stained with Coomassie blue to show secretion of mature ICAM-1 in E. coli under control of variant STII signal sequences. A TIR of relative strength 9 was provided by the pPho31 STII variant; a TIR of relative strength 3 was provided by the pPho41 STII variant. Precursor and mature forms of the polypeptide were identified.

In another case, a polypeptide gel was run and stained with Coomassie blue to show secretion of mature NT3 in E. coli under control of variant STII signal sequences. A TIR of relative strength 9 was provided by the pPho31 STII variant; a TIR of relative strength 7 was provided by the pPho21 STII variant; a TIR of relative strength 3 was provided by the pPho41

STII variant; the TIR of relative strength 1 was provided by the pPho51 STII variant. The mature form of the polypeptide was identified.

In still another case, a polypeptide gel was run and stained with Coomassie blue to show secretion of mature RANTES in E. coli under control of variant STII signal sequences. TIRs of relative strength 9 were provided by the pPho31 and the pSTBKPhoA#116 STII variants; a TIR of relative strength 7 was provided by the pPho21 STII variant; a TIR of relative strength 4 was provided by the pSTBKPhoA#81 STII variant; a TIR of relative strength 3 was provided by the pPho41 STII variant; a TIR of relative strength 2 was provided by the pSTBKPhoA#107 STII variant; TIRs of relative strength 1 were provided by the pSTBKPhoA#86 and the pPho51 STII variants. The mature form of the polypeptide was identified.--

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